

Green Synthesis of Zinc Oxide Nanoparticles Using Brown Seaweed *Padina boergesenii*: Characterization, Nutritional Profiling, Phytochemical Analysis, and Evaluation of Antibacterial Activity against Pathogenic Bacteria

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ABSTRACT

Background: Zinc Oxide Nanoparticles (ZnO NPs) offer broad-spectrum antibacterial activity, but conventional synthesis methods raise environmental and safety concerns. Green synthesis using marine algae like *Padina boergesenii* (*P. boergesenii*), rich in bioactive compounds, provides a sustainable and eco-friendly approach to nanoparticle production. **Objectives:** This study investigated the green synthesis of Zinc Oxide Nanoparticles (ZnO NPs) using the brown seaweed *P. boergesenii* and evaluated their antibacterial potential. **Materials and Methods:** ZnO NPs were synthesized using an aqueous extract of *P. boergesenii*. The nanoparticles were characterized by UV-visible spectroscopy, Fourier-Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD) analysis. Nutritional composition and phytochemical constituents of *P. boergesenii* were also determined. The antibacterial activity of the biosynthesized ZnO NPs was assessed against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas* sp. using a dose-dependent approach. **Results:** The biosynthesized nanoparticles were characterized using UV-vis spectroscopy, showing a characteristic absorption peak between 300-350 nm, confirming successful ZnO NP formation. FTIR analysis revealed the presence of biomolecules acting as reducing and capping agents, while XRD patterns demonstrated high crystallinity matching the standard (JCPDS No. 36-1451). Nutritional profiling of *P. boergesenii* revealed significant levels of carbohydrates (48.76±1.6%), ash (33.56±1.4%), moisture (74.62±2.3%), protein (5.42±0.6%), and lipids (1.9±0.2%). Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, phenols, cardiac glycosides, steroids, terpenoids, and quinones. Antibacterial assessment of the biosynthesized *P. boergesenii*-mediated ZnO NPs (Pb-ZnO NPs) demonstrated dose-dependent inhibition against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas* sp., with maximum activity observed at 100 µg/mL. *Staphylococcus aureus* showed higher susceptibility to the nanoparticles compared to the Gram-negative bacteria. **Conclusion:** These findings highlight the potential of *P. boergesenii* as a natural source for synthesizing ZnO NPs with promising applications in biomedical and pharmaceutical fields, particularly as antimicrobial agents against pathogenic bacteria.

Keywords: Zinc oxide nanoparticles, Green synthesis, *Padina boergesenii*, Antibacterial.

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INTRODUCTION

Marine algae represent a renewable and sustainable resource with significant potential for various applications in biotechnology, pharmaceuticals, and materials science. Among these, brown

seaweeds have garnered considerable attention due to their rich biochemical composition and bioactive compounds that exhibit numerous biological activities. *Padina boergesenii* (*P. boergesenii*), a brown macroalga belonging to the family Dictyotaceae, is widely distributed in tropical and subtropical coastal regions and has been traditionally used for its medicinal properties.^[1]

The emergence of antibiotic resistance among pathogenic bacteria has become a global health crisis, necessitating the exploration of alternative antimicrobial strategies.^[2,3] Metallic nanoparticles, particularly ZnO NPs, have emerged as promising



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candidates for combating bacterial infections due to their unique physicochemical properties and broad-spectrum antimicrobial activity.^[4] Conventional methods for nanoparticle synthesis often involve hazardous chemicals and energy-intensive processes, raising environmental concerns. Green synthesis using biological entities such as plant extracts, algae, and microorganisms offers an eco-friendly alternative that aligns with sustainable development goals.^[5]

The biosynthesis of nanoparticles using marine algae has several advantages over terrestrial plants, including higher yield, absence of seasonal variations, and the presence of unique bioactive compounds. Brown seaweeds, in particular, contain sulfated polysaccharides, polyphenols, and other secondary metabolites that can serve as effective reducing and stabilizing agents in nanoparticle synthesis.^[6] These biomolecules not only facilitate the formation of nanoparticles but also potentially enhance their biological activities through surface functionalization. The nutritional composition and phytochemical profile of the source organism play crucial roles in determining the efficacy of biosynthesized nanoparticles. *P. boergesenii* is known to contain various bioactive compounds, including polysaccharides, phenolic compounds, and terpenoids, which contribute to its medicinal properties.^[7] These compounds can influence the size, shape, stability, and biocompatibility of the synthesized nanoparticles, thereby affecting their biological applications. ZnO NPs exhibit antimicrobial activity through multiple mechanisms, including the generation of ROS, disruption of bacterial cell membranes, and interference with metabolic pathways.^[8] The effectiveness of these mechanisms depends on various factors, including nanoparticle size, shape, surface charge, and functionalization. Biosynthesized ZnO NPs often demonstrate enhanced antimicrobial activity compared to chemically synthesized counterparts due to the synergistic effects of the biological capping agents.^[9]

The present study aimed to biosynthesize ZnO NPs using *P. boergesenii* extract and characterize them using advanced analytical techniques, including UV-vis spectroscopy, FTIR, and XRD. Additionally, the nutritional composition and phytochemical profile of *P. boergesenii* were analyzed to understand their potential role in nanoparticle formation and biological activities. The antibacterial efficacy of the biosynthesized ZnO NPs was evaluated against three bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas* sp., representing both Gram-positive and Gram-negative bacteria. This study contributes to the growing body of knowledge on marine algae-mediated nanoparticle synthesis and their potential applications in biomedical and pharmaceutical fields, particularly as antimicrobial agents against pathogenic bacteria.

MATERIALS AND METHODS

Collection and Preparation of *P. boergesenii* Extract

Fresh samples of *P. boergesenii* were collected from the Gulf of Mannar, along the southeastern coast of India. The samples were thoroughly washed with distilled water to remove contaminants and debris. After air-drying to remove excess moisture, the seaweed samples were ground into a fine powder using a mortar and pestle. 10 g of the powdered seaweed were immersed in 100 mL of sterile deionized water to obtain the extract. The mixture was agitated vigorously and then filtered using a No. 1 filter paper. The resulting filtrate was refrigerated and preserved for subsequent analysis.

Green Synthesis of Zinc Oxide Nanoparticles

The green synthesis of ZnO NPs was performed using a carefully controlled procedure. 1 g of powdered seaweed was mixed with 100 mL of double-distilled water and heated to 100°C. Aqueous extract was filtered from the resulting mixture. A precursor solution of zinc sulphate was prepared separately in a flask at the same temperature. Under continuous stirring and maintaining the desired solution pH, the seaweed extract was slowly added drop by drop to the zinc sulphate solution. The prepared mixture was incubated with the intent of nucleation and growth of ZnO NPs. Afterwards, the mixture was centrifuged at 1500 rpm for 20 min to isolate the solid nanoparticle product.^[10] The final product was obtained after the purified ZnO NPs were dried at 80°C overnight.

Characterization of Zinc Oxide Nanoparticles

The synthesized ZnO NPs were characterized using multiple analytical techniques to determine their structural, optical, and surface properties. The optical properties were examined using UV-Visible spectroscopy at room temperature in the wavelength range of 200–800 nm, where a characteristic absorption peak was observed around 360–370 nm, corresponding to the band gap of ZnO. The surface functional groups and possible biomolecule interactions on ZnO NP surfaces were identified using Fourier Transform Infrared (FTIR) spectroscopy in the range of 400–4000 cm⁻¹ using the KBr pellet method. The crystalline structure and phase purity of the ZnO NPs were determined by X-ray diffraction operated at 40 kV and 30 mA.

Proximate Composition Estimation

The proximate composition of *P. boergesenii* was determined to assess its nutritional constituents. Moisture content was measured by drying the algal samples at 105 °C until a constant weight was achieved. Lipid content was estimated using the Soxhlet extraction method with petroleum ether as the solvent. Ash content was determined by incinerating the dried samples in a muffle furnace at 550 °C until a constant weight was obtained. Protein content was quantified using the Kjeldahl method. Total

carbohydrate content was calculated by difference using the formula: Carbohydrate (%) = 100 - (%Moisture + %Protein + %Lipid + %Ash).

Screening for Phytochemicals

Qualitative phytochemical analysis of the algal extracts was carried out following standard procedures to detect major secondary metabolites.^[11] The presence of alkaloids was confirmed by Mayer's test, where addition of Mayer's reagent produced a reddish-brown precipitate. Saponins were identified by the foam test, in which vigorous shaking of the extract with water resulted in persistent frothing. Tannins were detected by the appearance of a dark blue or greenish-black color upon treatment with ferric chloride solution. Cardiac glycosides were confirmed by the Keller–Killani test, which yielded a characteristic brown ring at the interface between glacial acetic acid–ferric chloride solution and concentrated sulphuric acid. Flavonoids were detected by the alkaline reagent test, showing an intense yellow color that disappeared on addition of dilute HCl. Phenolic compounds were identified by the formation of a bulky white precipitate with lead acetate solution. Steroids were indicated by a red coloration in the upper chloroform layer and a yellow-green fluorescence in the lower sulphuric acid layer. Terpenoids were confirmed by the Salkowski test, where a reddish-brown coloration developed at the interface of chloroform and concentrated sulphuric acid. Quinones were identified by the development of red to blue coloration upon treatment with alcoholic KOH. Proteins, peptides, or amino acids were detected by the ninhydrin test, where heating with freshly prepared reagent produced a pink or purple color.

Evaluation of Antibacterial Activity

The antibacterial activity of the *P. boergesenii*-mediated zinc oxide nanoparticles (Pb-ZnO NPs) was evaluated using the agar well diffusion method against pathogenic bacteria. Initially, cultures of each of the bacteria, namely, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas* species, were prepared in Muller Hinton broth. Then, the cultured bacteria were swabbed on the Muller Hinton agar plates. Wells of 6 mm diameter were created in agar plates. Then, different volumes (25, 50 and 100 µg/mL) of the Pb-ZnO NPs synthesized were added into the wells, including a standard antibiotic solution (30 µg/mL) for comparison. Thereafter, the plates were incubated for 24 hr to allow the interaction of nanoparticles with bacteria. The inhibition zone diameters around each well were measured after incubation to determine the antibacterial efficiency of the Pb-ZnO NPs against bacterial strains under test.

Statistical Analysis

All experiments were conducted in triplicate, and data were presented as Mean ± Standard Deviation. Statistical analysis was

performed to evaluate the significance of observed differences using appropriate software packages.

Ethical Consideration and Patient Consent

This study did not require ethical approval and patient consent as it does not involve human participants or animal subjects.

RESULTS

Characterization of Biosynthesized ZnO Nanoparticles

The biosynthesized ZnO NPs from *P. boergesenii* were characterized using UV-vis spectroscopy, FTIR, and XRD analyses to confirm their formation, stability, functional groups, and crystallinity. The UV-vis absorption spectrum (Figure 1A) exhibited a strong absorbance in the UV region, with a peak around 300-350 nm, which is a characteristic feature of ZnO nanoparticles due to excitonic transitions. The sharp absorption edge indicates the successful synthesis of ZnO, while the gradual decrease in absorbance beyond 400 nm suggests minimal agglomeration, ensuring nanoparticle stability. The presence of a well-defined absorption peak also provides insights into the optical band gap of ZnO nanoparticles, which is crucial for their photocatalytic and biomedical applications.

The FTIR spectrum (Figure 1B) revealed distinct peaks corresponding to functional groups responsible for nanoparticle formation and stabilization. Peaks in the range of 1000-1700 cm⁻¹ suggest the presence of biomolecules such as polyphenols, proteins, and polysaccharides, which play a crucial role in reducing and capping the ZnO nanoparticles. The presence of hydroxyl (-OH) and carbonyl (C=O) stretching vibrations further confirms the interaction between plant metabolites and the ZnO surface. These biomolecules not only act as stabilizing agents but also enhance the biocompatibility of the synthesized nanoparticles, making them suitable for biomedical applications such as antimicrobial and anti-inflammatory treatments.

The XRD pattern (Figure 1C) confirmed the crystalline nature of the ZnO nanoparticles, with sharp and well-defined diffraction peaks at 2θ values corresponding to (220), (311), (400), (440), and (511) planes. The observed peaks match the standard ZnO wurtzite phase (JCPDS No. 36-1451), confirming the purity and phase integrity of the nanoparticles. The high-intensity peaks indicate excellent crystallinity, which is a key factor influencing the physicochemical properties of ZnO, such as surface reactivity and stability. The absence of additional impurity peaks suggests that the biosynthesis method using *P. boergesenii* produces pure ZnO nanoparticles without secondary phases.

Proximate Composition and Phytochemical Screening

The proximate analysis of *P. boergesenii* (Table 1) revealed its nutritional composition, which includes moisture, carbohydrates,

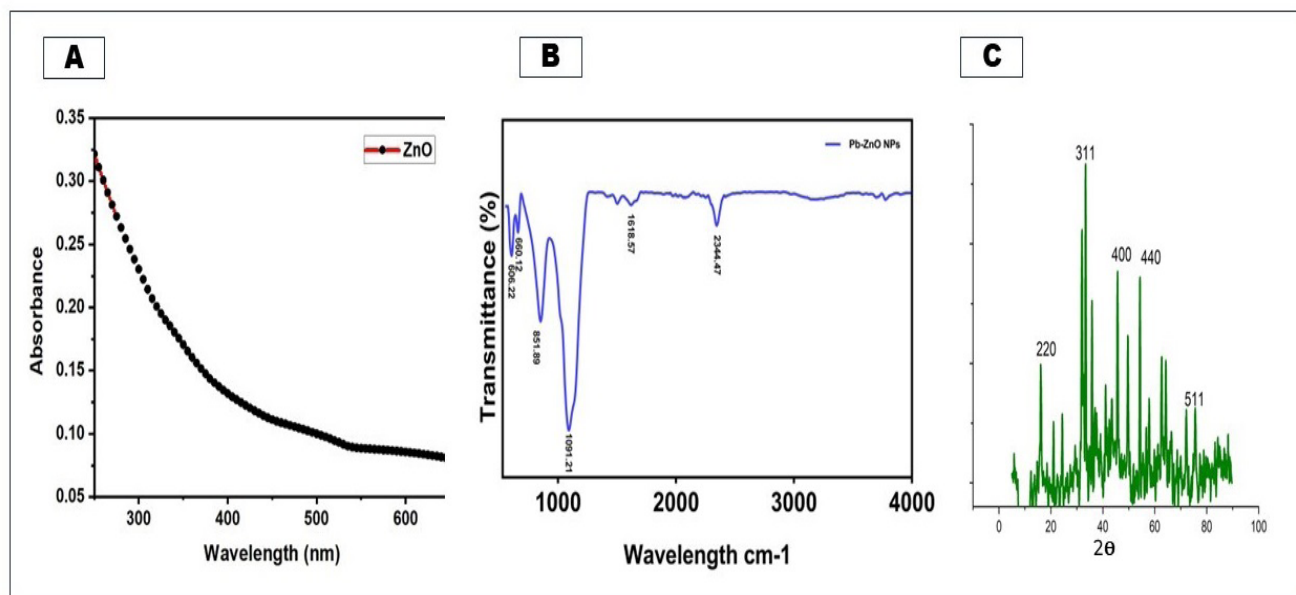


Figure 1: Characterization of biosynthesized ZnO nanoparticles from *P. boergesenii*. (A) UV-vis absorption spectrum showing a strong absorbance in the UV region. (B) FTIR spectrum indicating the presence of functional groups responsible for the reduction and stabilization of ZnO nanoparticles. (C) XRD pattern confirming their crystalline nature with characteristic peaks corresponding to ZnO.

ash content, lipids, and proteins. These components play a crucial role in determining its potential applications in pharmaceuticals, nutraceuticals, and biomedical research. The moisture content of *P. boergesenii* was recorded as $74.62 \pm 2.3\%$, indicating its high-water retention capacity. This aligns with the characteristics of marine algae, which typically have high moisture levels due to their aquatic habitat. A high moisture content can influence the shelf life and storage stability of the biomass, necessitating proper drying and preservation techniques for further applications. The carbohydrate content was found to be $48.76 \pm 1.6\%$, making it a major component of *P. boergesenii*. Carbohydrates in marine algae mainly consist of polysaccharides, including alginates, laminarin, and fucoïdan, which are known for their biological activities such as antioxidant, anticoagulant, and immune-modulating properties. The high carbohydrate content suggests the potential use of *P. boergesenii* in the production of bioactive polysaccharides for pharmaceutical applications, including drug delivery and wound healing formulations. The ash content was recorded as $33.56 \pm 1.4\%$, which indicates the presence of significant amounts of minerals and inorganic components. Marine algae are rich sources of essential minerals, including calcium, magnesium, potassium, and trace elements such as zinc and iron. These minerals contribute to various physiological functions and can enhance the nutritional and therapeutic value of *P. boergesenii*. The high ash content further supports its potential for use in mineral supplementation and nutraceutical applications. The lipid content was relatively low at $1.9 \pm 0.2\%$, which is consistent with the general composition of brown algae, which are not typically rich in fats. However, the lipids present may include bioactive

Table 1: Moisture, ash, carbohydrate, protein, and fat concentrations in *P. boergesenii* (Mean \pm Standard deviation).

Content	<i>P. boergesenii</i> %
Moisture	74.62 ± 2.3
Carbohydrate	48.76 ± 1.6
Ash	33.56 ± 1.4
Lipid	1.9 ± 0.2
Protein	5.42 ± 0.6

Values are articulated as Mean \pm S.D ($n=3$).

compounds such as polyunsaturated fatty acids and sterols, which have anti-inflammatory and cardioprotective properties. Despite its low lipid content, *P. boergesenii* may still serve as a source of beneficial marine-derived lipids for pharmaceutical formulations. The protein content was measured at $5.42 \pm 0.6\%$, indicating a moderate presence of proteinaceous compounds. While *P. boergesenii* is not a primary source of proteins, the proteins it does contain may include bioactive peptides with antioxidant, antimicrobial, and enzyme-inhibitory properties. The moderate protein content suggests that *P. boergesenii* may contribute to dietary protein intake, but its primary value lies in its secondary metabolites rather than its protein content. As shown in Table 1, the moisture content of *P. boergesenii* was found to be $74.62 \pm 2.3\%$, indicating a high-water content typical of marine algae. The carbohydrate content, at $48.76 \pm 1.6\%$, suggests a significant source of energy and potential prebiotic compounds. The high ash content ($33.56 \pm 1.4\%$) is indicative of a rich mineral profile, which is characteristic of marine algae. The lipid and protein contents were relatively low at $1.9 \pm 0.2\%$ and $5.42 \pm 0.6\%$, respectively.

Phytochemical Screening of *P. boergesenii*

The phytochemical screening of *P. boergesenii* extract confirmed the presence of several bioactive compounds, including alkaloids, flavonoids, saponins, phenols, cardiac glycosides, steroids, terpenoids, and quinones, while tannins and proteins were absent as mentioned Table 2 and Figure 2. These findings suggest that *P. boergesenii* is a rich source of secondary metabolites, which contribute to its biomedical potential, particularly in antimicrobial, antioxidant, and anti-inflammatory applications. The presence of alkaloids indicates that *P. boergesenii* may possess antibacterial, antifungal, and cytotoxic properties, as alkaloids are known for their role in disrupting microbial cell functions and inhibiting cancer cell proliferation. Flavonoids, which exhibited a positive reaction, are well known for their strong antioxidant activity, which can help neutralize free radicals, reducing oxidative stress-related diseases such as inflammation and cancer. Their presence suggests that *P. boergesenii* could serve as a natural antioxidant source for therapeutic applications. The detection of saponins, indicated by froth formation, is significant because saponins exhibit antimicrobial, hemolytic, and immune-modulating properties. They can enhance the permeability of bacterial membranes, making them effective against pathogenic microbes. Additionally, phenols, detected through colorimetric changes, are potent antioxidants that can protect cells from oxidative damage, further supporting the biomedical applications of *P. boergesenii*. The presence of cardiac glycosides suggests potential cardioprotective activity, as these compounds are known for their ability to regulate heart function by influencing sodium-potassium ion exchange in cardiac muscles. Their presence in *P. boergesenii* adds to its pharmacological relevance. Steroids and terpenoids, identified through distinct layered formations, play essential roles in anti-inflammatory and cytotoxic activities. Steroids contribute to membrane stabilization, while terpenoids have been reported to possess antimicrobial, anti-inflammatory, and anticancer properties. Quinones, which exhibited a positive result, are known for their antimicrobial and anticancer effects due to their ability to generate ROS and interfere with microbial metabolism. Their presence suggests that *P. boergesenii* could be a valuable natural source for antimicrobial formulations. On the other hand, tannins and proteins were absent in the extract, which may indicate that *P. boergesenii* has a lower astringency compared to tannin-rich plants. The absence of proteins suggests that the biological activity of the extract is primarily mediated by secondary metabolites rather than protein-based compounds.

Antibacterial Activity of Pb-ZnO NPs

The antibacterial activity of *P. boergesenii*-derived ZnO NPs was evaluated against three bacterial strains: *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Pseudomonas* sp. using the

well diffusion method. Table 3 presents the Zone of Inhibition (ZOI) at different ZnO NP concentrations (25 µg/mL, 50 µg/mL, and 100 µg/mL), along with a control (C) that did not show any significant inhibition. The results confirm a dose-dependent antibacterial activity, where higher concentrations of ZnO NPs resulted in larger inhibition zones.

Against *Pseudomonas* sp., the inhibition zones increased with concentration, with 100 µg/mL exhibiting the largest zone (Table 3). The control (C) showed no inhibition, confirming that the antibacterial effect was due to ZnO NPs. Compared to *S. aureus*, the inhibition zones for *Pseudomonas* sp. were smaller, suggesting moderate sensitivity to ZnO NPs. The ability of ZnO NPs to inhibit *Pseudomonas* sp. growth highlights their potential in controlling infections caused by this pathogen, which is known for its antibiotic resistance.

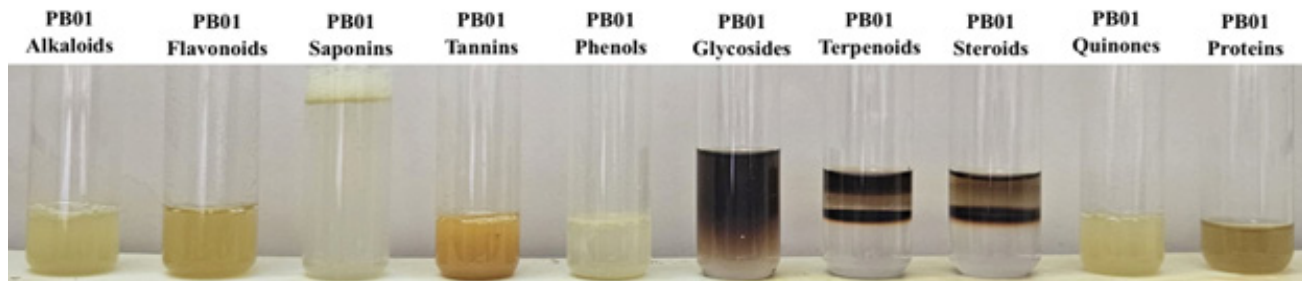
For *Staphylococcus aureus*, the bacterial lawn showed clear inhibition zones, with the highest concentration (100 µg/mL) producing the largest zone (Table 3). The inhibition zones for *S. aureus* were larger compared to *E. coli* and *Pseudomonas* sp., indicating that it is more susceptible to ZnO NPs. The increased sensitivity of *S. aureus* can be attributed to its Gram-positive nature, as it lacks an outer membrane, making it more vulnerable to ZnO NP-induced oxidative stress and membrane disruption. This finding suggests that *P. boergesenii*-ZnO NPs could be effective against Gram-positive bacterial infections. Against *E. coli*, the inhibition zones were present but relatively smaller compared to *S. aureus* (Table 3). As a Gram-negative bacterium, *E. coli* possesses an outer membrane that may reduce the penetration of ZnO NPs, leading to a lower antibacterial effect. However, a dose-dependent response was still observed, with 100 µg/mL showing the highest inhibition. This suggests that while *E. coli* is less susceptible than *S. aureus*, ZnO NPs still exhibit antibacterial activity against it, making them a potential alternative to conventional antibiotics for treating infections caused by Gram-negative bacteria.

Table 2: Qualitative examination of ethanolic extract; ("+" indicates presence and "-" indicates absence).

Content	<i>P. boergesenii</i>
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	-
Phenols	+
Cardiac glycosides	+
Steroids	+
Terpenoids	+
Quinones	+
Proteins	-

Table 3: Zone of inhibition (mm) at different concentrations ($\mu\text{g/mL}$) of Pb-ZnO NPs.

<i>P. boergesenii</i> ZnO NPs	25	50	100	C
<i>E. coli</i>	12	13	15	9
<i>S. aureus</i>	17	21	24	9
<i>Pseudomonas</i> sp.	12	14	18	9

**Figure 2:** Qualitative phytochemical analysis of *P. boergesenii* extract.

DISCUSSION

The present study demonstrated the successful biosynthesis of ZnO NPs using the brown seaweed *P. boergesenii*. The characterization, nutritional analysis, phytochemical screening, and antibacterial evaluation collectively provide valuable insights into the potential applications of these marine algae-mediated nanoparticles. The UV-Vis spectroscopic analysis revealed a characteristic absorption peak in the range of 300-350 nm, which is consistent with previous reports on ZnO NPs.^[12,13] This absorption peak is attributed to the excitonic transitions of ZnO, confirming the successful formation of nanoparticles. The sharp absorption edge indicates minimal agglomeration, suggesting that the biomolecules present in *P. boergesenii* extract effectively acted as stabilizing agents.^[13] These results are consistent with previous reports demonstrating comparable spectroscopic characteristics of ZnO NPs synthesized through green synthesis approaches.^[14] The FTIR analysis provided valuable information about the functional groups involved in the reduction and stabilization of ZnO nanoparticles.^[15] The presence of peaks corresponding to hydroxyl (-OH) and carbonyl (C=O) stretching vibrations indicates the involvement of polyphenols, proteins, and polysaccharides in the biosynthesis process. These biomolecules act as natural capping agents, enhancing the stability and biocompatibility of the nanoparticles.^[16] Similar findings have shown that plant metabolites play a vital role in the green synthesis of metal nanoparticles.^[17] The XRD patterns confirmed the crystalline nature of the biosynthesized ZnO NPs, with characteristic peaks matching the standard wurtzite phase.^[18] The high-intensity peaks suggest excellent crystallinity, which is essential for the physicochemical properties of ZnO, including surface reactivity and stability.^[19] The absence of impurity peaks indicates the purity of the synthesized nanoparticles, highlighting the efficiency of the green synthesis approach using *P. boergesenii*. These findings are consistent with those of Anand *et al.*, 2024 who

reported similar XRD patterns for ZnO nanoparticles synthesized using marine algae.^[20]

The nutritional analysis of *P. boergesenii* revealed a rich biochemical composition, with significant levels of carbohydrates ($48.76 \pm 1.6\%$), ash ($33.56 \pm 1.4\%$), moisture ($74.62 \pm 2.3\%$), protein ($5.42 \pm 0.6\%$), and lipids ($1.9 \pm 0.2\%$). The high carbohydrate content suggests the presence of polysaccharides, such as alginates, laminarin, and fucoidan, which are known for their reducing and stabilizing properties in nanoparticle synthesis.^[21] These polysaccharides can form coordination bonds with metal ions, facilitating the formation of stable nanoparticles. The high ash content indicates a rich mineral profile, which could contribute to the bioactivity of the synthesized nanoparticles. Marine algae typically accumulate various minerals, including zinc, which may enhance the formation of ZnO nanoparticles. The moderate protein content suggests the presence of amino acids and peptides, which can act as reducing agents through their electron-rich functional groups.^[22]

The phytochemical screening revealed the presence of diverse bioactive compounds, including alkaloids, flavonoids, saponins, phenols, cardiac glycosides, steroids, terpenoids, and quinones. These secondary metabolites play crucial roles in the biosynthesis of nanoparticles. Phenolic compounds, such as flavonoids, are known for their strong antioxidant properties and can reduce metal ions through electron donation.^[23] Similarly, terpenoids can form intermediate complexes with metal ions, leading to nanoparticle formation. The absence of tannins and proteins in the extract suggests that the reducing and stabilizing properties of *P. boergesenii* are primarily attributed to other phytochemicals. This finding highlights the complex interplay of various biomolecules in the green synthesis of nanoparticles. The rich phytochemical profile of *P. boergesenii* not only facilitates nanoparticle formation but also potentially contributes to the enhanced biological activities of the synthesized nanoparticles.^[24]

The biosynthesized *P. boergesenii*-mediated ZnO NPs demonstrated significant antibacterial activity against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli* and *Pseudomonas* sp.) bacteria.^[25] The observed dose-dependent inhibition, with maximum activity at 100 µg/mL, suggests that the nanoparticles provide their antibacterial effects in a concentration-dependent manner. This finding is consistent with previous studies reporting the concentration-dependent antibacterial activity of ZnO NPs. The differential susceptibility of the tested bacterial strains to Pb-ZnO NPs can be attributed to structural differences in their cell walls. *S. aureus*, being a Gram-positive bacterium, lacks an outer membrane and possesses a thick peptidoglycan layer, making it more susceptible to nanoparticle-induced damage.^[26] In contrast, Gram-negative bacteria (*E. coli* and *Pseudomonas* sp.) have an additional outer membrane composed of lipopolysaccharides, which provides a protective barrier against external agents.^[27] Future research should focus on elucidating the exact mechanisms of antibacterial action of Pb-ZnO NPs, investigating their potential synergistic effects with conventional antibiotics, and evaluating their safety and efficacy in *in vivo* models. Additionally, exploring the applications of these nanoparticles in food preservation, wound healing, and targeted drug delivery could expand their utility in various fields.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

P. boergesenii: *Padina boergesenii*; **ZnO NPs**: Zinc oxide Nanoparticles; **XRD**: X-ray Diffraction; **FTIR**: Fourier Transformer Infrared; **ROS**: Reactive Oxygen Species; **Pb-ZnO NPs**: *P. boergesenii* - Derived Zinc Oxide Nanoparticles.

SUMMARY

The present study successfully demonstrated the green synthesis of Zinc oxide nanoparticle (ZnO NPs) using *P. boergesenii*, confirming their crystalline nature and stability through UV-vis, FTIR, and XRD characterization. The biosynthesized ZnO NPs exhibited remarkable antibacterial efficacy in a concentration-dependent manner, with *S. aureus* displaying the highest zone of inhibition (17 mm at 25 µg/mL to 24 mm at 100 µg/mL), followed by *Pseudomonas* sp. (12 mm to 18 mm) and *E. coli* (12 mm to 15 mm), while the control exhibited minimal inhibition (9 mm). This suggests the potential of *P. boergesenii*-ZnO NPs as an effective antimicrobial agent against both Gram-positive and negative bacteria. Given their strong antibacterial potential, these biosynthesized *P. boergesenii*-ZnO NPs hold significant promise for biomedical applications, particularly in antimicrobial therapies. Future studies should focus on mechanistic insights, *in*

in vivo evaluations, and formulation development to facilitate their translation into clinical applications.

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